Journal of Medical Virology 53:157–161 (1997)

Infection With GB Virus C and Hepatitis C Virus in Drug Addicts, Patients on Maintenance Hemodialysis, or With Chronic Liver Disease in Nepal

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Infection with GB virus C (GBV-C) and hepatitis C virus (HCV) was surveyed in various populations in Kathmandu, Nepal. GBV-C RNA and HCV RNA were detected in four (2%) and none, respectively, of 181 normal controls. Viral RNAs were detected significantly more frequently ($P < 0.001$) in 32 (44%) and 43 (60%), respectively, of 72 users of illicit intravenous drug, and in three (14%) and one (5%) of 22 patients on maintenance hemodialysis. The three hemodialysis patients with GBV-C RNA had been transfused with more blood units than the 19 without GBV-C RNA (51 ± 21 vs. 5 ± 3 units, $P < 0.01$), and one was co-infected with HCV. Of 145 patients with chronic liver disease, GBV-C RNA was detected in four (3%) and HCV RNA in 12 (8%); only one patient with GBV-C RNA was without markers of HCV or hepatitis B virus infection. In the 32 drug addicts infected with GBV-C, genotypes were G1 in two (6%), G2 in 26 (81%), G3 in three (9%), and the remaining one (3%) was coinfected with G2 and G3. GBV-C genotypes in the 13 individuals in the populations other than drug addicts were G2 in 11 (85%) and G3 in two (15%). HCV genotypes in the 43 drug addicts with viremia were I/1a in 21 (49%), V/3a in 19 (44%) and I/1a plus V/3a in two (5%); these genotypes were not prevalent in normal controls and patients with chronic liver disease in Nepal. These results indicate that GBV-C infection is prevalent in healthy subjects in Nepal at a frequency (2%) comparable with those in the other countries and that GBV-C transmits efficiently by intravenous drug abuse among drug addicts and by transfusion in hemodialysis patients. ***J. Med. Virol. 53:157–161, 1997***

KEY WORDS: hepatitis viruses; hepatitis C viruses; intravenous drug abuse; hemodialysis; chronic liver disease

INTRODUCTION

A viral agent presumably responsible for a portion of non-A to E hepatitis has been reported independently by two groups of investigators and named GB virus C (GBV-C) and hepatitis G virus (HGV), respectively [Simons et al., 1995; Leary et al., 1996; Linnen et al., 1996]. They both belong to *Flaviviridae* and share >86% of nucleotides and >96% of amino acid sequences. Therefore, they are considered to be separate isolates of the same virus, referred to here as GBV-C collectively.

GBV-C is a highly adapted human virus, infecting 1–2% or more of apparently healthy individuals worldwide [Dawson et al., 1996; Fiordalisi et al., 1996; Heringlake et al., 1996; Linnen et al., 1996; Masuko et al., 1996; Brown et al., 1997; Wang et al., 1997]. Although the disease-inducing activity of GBV-C is not clear as yet, the viral RNA is detected more frequently in patients with acute or chronic non-A to E hepatitis than in controls [Dawson et al., 1996; Fiordalisi et al., 1996]. GBV-C frequently co-infects with hepatitis viruses such as hepatitis C virus (HCV) and hepatitis B virus

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Accepted 4 June 1997

(HBV) [Simons et al., 1995; Linnen et al., 1996; Masuko et al., 1996; Tsuda et al., 1996]. It transmits parenterally typified by transfusions and illicit intravenous drugs [Aikawa et al., 1996; Masuko et al., 1996; Wang et al., 1996; Alter et al., 1997].

We surveyed various populations in Nepal, for GBV-C RNA and markers of HCV and HBV. GBV-C RNA was detected in 2% of normal controls, which was as frequent as in other countries and was very prevalent at 44% among drug addicts.

MATERIALS AND METHODS

Studied Subjects

During February through September, 1996, serum samples were obtained in Nepal from 72 drug addicts admitted to a rehabilitation center in the suburbs of Kathmandu and 41 patients with chronic renal failure, including 22 on maintenance hemodialysis at Bir hospital, a city facility in Kathmandu, as well as 181 apparently healthy individuals undergoing routine checkups. The 145 patients with chronic liver disease at Bir hospital, including 20 with chronic hepatitis, 63 with liver cirrhosis, and 62 with hepatocellular carcinoma, as well as 49 carriers of hepatitis B surface antigen (HBsAg) identified among blood donors and staff members of Bir hospital, have been reported previously [Shrestha et al., 1994].

Detection of GBV-C RNA

GBV-C RNA was determined by reverse-transcription polymerase chain reaction (RT-PCR) with primers deduced from the 5′ untranslated region (UTR) of the GBV-C genome by the method described previously [Shimizu et al., 1996].

GBV-C Genotypes

Three genotypes of GBV-C, provisionally designated G1, G2, and G3 [Okamoto et al., 1997], were determined by selective amplification with type-specific primers deduced from the 5′UTR [Fukushi et al., 1996; Leary et al., 1996; Linnen et al., 1996; Muerhoff et al., 1996; Shao et al., 1996; Okamoto et al., 1997]. Products of the first round PCR for the detection of GBV-C RNA, with universal primers G58 (sense) sequenced 5′-CAG GGT TGG TAG GTC GTA AAT CC-3′ and G75 (antisense) with a sequence of 5′-CCT ATT GGT CAA GAG AGA CAT-3′, were amplified with primers specific for each of the three genotypes in two separate reactions.

Reaction one was carried out with a universal primer (G134: sense), which was sequenced 5′-GGT CAY CYT GGT AGC CAC TAT AGG-3′ (Y = T or C) and used in the second-round PCR for the detection of GBV-C RNA, and two antisense primers specific for G1 (G144: 5′-TTT AAC GGC GTG CCT AG<u>G</u> GC-3′) [underlined is an intentional mismatch from C to G for increasing the specificity]) or G2 (G143: 5′-GCC GCA GGC ACA AGA G<u>C</u>A AT-3′ [a mismatch from G underlined]). The reaction generated an amplification product of 139 base pairs (bp) for G1 genotype and that of 66 bp for G2 genotype.

Reaction two was undertaken with two sense primers specific for G1 (G140: 5′-GGA CCC GGC GCT AGG CA<u>G</u> GC-3′ [a mismatch from C underlined]) or G3 (G167: 5′-AAG GTC CCT CTR GCG CT<u>A</u> GT-3′ [R = G or A; a mismatch from T underlined]) and a universal primer (G131: antisense) that was sequenced 5′-AAG AGA GAC ATT GWA GGG CGT-3′ (W = T or A) and used in the second-round PCR for the detection of GBV-C RNA. The reaction amplified a product of 165 bp for G3 genotype and that of 96 bp for G1 genotype; the latter could confirm the G1 genotype determined in Reaction one.

PCR was carried out for 25 cycles with 94 °C, 30 sec; 60 °C, 30 sec; 72 °C, 30 sec (7 min in the last cycle).

Markers of Other Viruses

Antibody to HCV (anti-HCV) was detected by a second-generation enzyme immunoassay (HCV ELISA II, Ortho Diagnostic Systems, Tokyo, Japan) and an enzyme immunoassay with synthetic HCV core peptides (anti-HCV core) with commercial kits (SMITEST HCV Core Ab ELISA, Sumitomo Metal Industries, Tokyo, Japan). Samples reactive with either or both assays were considered positive for anti-HCV. Sera positive for anti-HCV were tested for HCV RNA by RT-PCR with commercial kit (Amplicor HCV detection kit, Japan Roche, Tokyo, Japan). The five common HCV genotypes designated by the mixed nomenclature (I/1a, II/1b, III/2a, IV/2b, and V/3a) were determined by selective amplification by type-specific primers deduced from the core gene [Okamoto et al., 1996]

HBsAg and the corresponding antibody (anti-HBs) were determined by passive hemagglutination with commercial kits (MyCell, Institute of Immunology Co., Tokyo, Japan). Antibody to hepatitis B core (anti-HBc) was determined by hemagglutination inhibition by the method described elsewhere [Iizuka et al., 1992]. Sera positive for anti-HBs, anti-HBc, or both were considered to contain antibody to HBV (anti-HBV).

Antibody to the human immunodeficiency virus type 1 (anti-HIV) was determined by particle agglutination with commercial kits (GENEDIA·HIV-1/2 Mix PA Auto and SERODIA·HIV, Fujirebio Diagnostics, Tokyo, Japan).

Statistical Analyses

Frequency between groups was compared using χ^2 test, and Fisher's exact test. Group means were compared using the Wilcoxon rank-sum test.

RESULTS

Infection With GBV-C, HCV, and HBV Among Various Populations in Nepal

Table I compares prevalence rates of GBV-C RNA as well as markers of HCV and HBV infections among various groups in Nepal. GBV-C RNA was detected in four of 181 (2%) apparently healthy individuals serving as normal controls. Anti-HCV was detected in two (1%), but HCV RNA was detected in none of them; only one (0.6%) was positive for anti-HIV. Of the four indi-

TABLE I. Markers of Hepatitis Virus Infection in Various Populations in Nepal

Populations	N	Age (years)	Male	GBV-C RNA	Anti-HCV	HCV[a] RNA	HBsAg	Anti-HBV[b]
Drug addicts	72	27 ± 7	71 (99%)	32 (44%)	58 (81%)	43 (60%)	2 (3%)	44 (61%)
Chronic renal failure	41	45 ± 16	26 (63%)	3 (7%)	2 (5%)	1 (2%)	1 (2%)	6 (15%)
Chronic liver disease	145	49 ± 16	112 (77%)	4 (3%)	13 (9%)	12 (8%)	57 (39%)	74 (51%)
HBV carriers	49	29 ± 9	44 (90%)	2 (4%)	1 (2%)	0	49 (100%)	49 (100%)
Normal controls	181	30 ± 14	103 (57%)	4 (2%)	2 (1%)	0	9 (5%)	46 (25%)
Differences[c]				$P < 0.001$	$P < 0.001$	$P < 0.001$		$P < 0.001$

[a]Tested only in sera positive for anti-HCV.
[b]Anti-HBs, anti-HBc, or both.
[c]Evaluated for drug addicts against normal controls.

viduals with GBV-C RNA, one was positive for anti-HBV and anti-HIV; markers of HCV, HBV or HIV were not detected in the remaining three.

GBV-C RNA was detected in 32 (44%) and HCV RNA in 43 (60%) of 72 users of illicit intravenous drugs, significantly more frequently ($P < 0.001$) than in 2% and 1%, respectively, of 181 normal controls. Anti-HBV was detected in 61% of them at a frequently higher than in 25% of normal controls ($P < 0.001$); the frequency of HBsAg was comparable between the two populations (3% vs. 5%). Four of the 72 (6%) drug addicts tested positive for anti-HIV.

GBV-C RNA was detected in three of 41 (7%) patients with chronic renal failure and two of 49 (4%) carriers of HBsAg, a little more frequently than in normal controls (2%).

GBV-C RNA was detected in four of 145 (3%) patients with chronic liver disease. They included one of the 20 (5%) patients with chronic hepatitis, one of the 63 (2%) patients with liver cirrhosis, and two of the 62 (3%) patients with hepatocellular carcinoma. Of the four patients with GBV-C RNA, one with hepatocellular carcinoma was without markers of HCV or HBV infection, whereas the other three patients were with HBV-associated liver disease. All the four patients with GBV-C RNA were negative for anti-HCV. HBsAg was detected in 57 (39%) and HCV RNA in 12 (8%), leaving 76 patients with an unknown etiology of liver disease, of whom GBV-C RNA was detected in only one (1%).

Markers of Hepatitis Virus Infections in Drug Addicts With or Without GBV-C RNA

Table II compares markers of HCV and HBV infections, as well as anti-HIV, between the 32 drug addicts with GBV-C RNA and the 40 without it. Anti-HCV was detected more frequently in the drug addicts with GBV-C RNA than in those without (94% vs. 70%, $P < 0.05$). There were no differences in the prevalence rates of the other viral markers. The distribution of HCV genotypes was comparable between the drug addicts with and without GBV-C RNA.

GBV-C RNA in Hemodialysis Patients

Of the 41 patients with chronic renal failure, 22 were on maintenance hemodialysis and all of them had received transfusions. Three of the 22 hemodialysis patients (14%) had GBV-C RNA and one (5%) possessed HCV RNA. GBV-C RNA or HCV RNA was not detected in any of the 19 patients who had not received transfusion or hemodialysis. Anti-HBV was found in three of the 22 (14%) patients on hemodialysis and a history of transfusion, which was comparable with the detection of anti-HBV in three of the 19 (16%) patients without transfusions or hemodialysis.

TABLE II. Markers of Hepatitis Virus Infections in Drug Addicts With or Without GBV-C RNA

	CBV-C RNA (+) (N = 32)	CBV-C RNA (−) (N = 40)	Differences
Age (years)	26 ± 5	29 ± 8	
Anti-HCV	30 (94%)	28 (70%)	$P < 0.05$
HCV RNA	22 (69%)	21 (52%)	
HCV genotypes			
I/1a	13 (41%)	8 (20%)	
V/3a	7 (22%)	12 (30%)	
I/1a plus V/3a	2 (6%)	0	
Unclassified	0	1 (3%)	
HBsAg	1 (3%)	1 (3%)	
Anti-HBV	20 (63%)	24 (60%)	
Anti-HIV	2 (6%)	2 (5%)	

The hemodialysis patients with GBV-C RNA and those without are compared in Table III. The three patients with GBV-C RNA had received more units of transfusions than the 19 without GBV-C RNA ($P < 0.01$). Although the patients with GBV-C RNA had received hemodialysis for more times than those without it, the difference fell short of being significant. HCV RNA was detected in a single patient who was co-infected with GBV-C.

Genotypes of GBV-C

The three genotypes of GBV-C designated G1, G2, and G3 [Okamoto et al., 1997] were determined in infected persons in various populations. The G1 genotype was not detected in any of the 13 individuals with GBV-C RNA in the populations other than drug addicts. G2 accounted for the majority and detected in 11 (85%) among them. G3 was detected in the remaining two (15%); they were a patient with chronic liver disease and a patient on maintenance hemodialysis.

Of the 72 drug addicts, 32 (44%) had GBV-C RNA and 43 (60%) possessed HCV RNA, and 22 of the 32 with GBV-C (69%) were co-infected with HCV. The G1

TABLE III. Comparison of Hemodialysis Patients With or Without GBV-C RNA

	GBV-C RNA (+) (N = 3)	GBV-C RNA (−) (N = 19)	Differences
Age (years)	39 ± 14	51 ± 15	
Male	0	15 (79%)	
Renal disease			
Diabetic nephropathy	2 (67%)	7 (37%)	
Glomerulonephritis	1 (33%)	6 (32%)	
Unknown	0	6 (32%)	
Transfusion (units)	51 ± 21	5 ± 3	$P < 0.01$
Hemodialysis (courses)	158 ± 81	77 ± 71	
Anti-HCV	1 (33%)	1 (5%)	
HCV RNA	1 (33%)	0	
HBsAg	0	0	
Anti-HBV	0	3 (16%)	

genotype of GBV-C was detected in two (6%), G2 in 26 (81%) and G3 in three (9%) of the 32 drug addicts infected with GBV-C. The remaining one drug addict was doubly infected with G2 and G3 genotypes.

Genotypes of GBV-C are shown for drug addicts in reference to genotypes of HCV infecting them (Table IV). G1 was detected only in the drug addicts infected with HCV of genotype I/1a.

DISCUSSION

GBV-C RNA was detected in four of 181 apparently healthy individuals (2%) who were living in Kathmandu, Nepal, and received routine health check-ups. GBV-C, therefore, seems to have been penetrated deeply into humans, infecting even the inhabitants of an area at a high altitude and segregated from the other parts of the world for many centuries.

GBV-C RNA was more frequent, however, in persons at increased risk for infection, such as drug addicts and patients on maintenance hemodialysis. Remarkably, GBV-C RNA was detected in 32 of the 72 (44%) habitual users of illicit intravenous drugs. It has been suggested that GBV-C should be classified into three distinct genotypes with divergence >12% either in the entire nucleotide sequence [Okamoto et al., 1997], or subgenomic regions [Muerhoff et al., 1996]. Genotype G2 was most prevalent accounting for the majority of infected individuals in Nepal. By contrast, genotype G1 was detected in two of the 32 (6%) drug addicts with viremia, but not in the 13 viremic persons in the other populations, including patients on maintenance hemodialysis or with chronic liver disease, normal controls, and HBsAg carriers.

Of the 72 drug addicts, 43 (60%) possessed HCV RNA, and 22 (51%) of them were co-infected with GBV-C. Genotypes of HCV in 43 drug addicts were I/1a in 21 (49%) and V/3a in 19 (44%). HCV genotypes in the 12 patients with chronic liver disease with HCV RNA have been reported previously [Shrestha et al., 1994]. They are I/1a in one (8%), II/1b in five (42%), V/3a in one (8%), and unclassifiable into the five common genotypes in the remaining five (42%). These unclassifiables have turned out to be rare genoytpes in genetic group 3 (one each of 3b, 3c, 3d, 3e, and 3f) in a later study [Tokita et al., 1994]. Hence the distribution of HCV genotypes were much different between drug addicts and patients with chronic liver disease in Nepal.

Taken together with the fact that the G1 genotype of GBV-C in the two drug addicts was accompanied by HCV of genotype I/1a in both, there would be a common route for GBV-C and HCV infections through intravenous drug abuse in Nepal. The rarity of G1 genotype of GBV-C as well as I/1a and V/3a genotypes of HCV among the general population in Nepal, taken along with their high frequency in drug addicts, would point to GBV-C and HCV of foreign origins prevailing in illicit intravenous drug abusers there. In order to substantiate this hypothesis, it would be necessary to determine GBV-C sequences in drug addicts and compare them with those in the other populations in Nepal in an extended survey. The determination of GBV-C sequences has been useful in verifying a foreign origin in Japanese hemophiliacs who had received inadequately sterilized blood products in the past [Kinoshita et al., 1997].

Of the 145 patients with chronic liver disease, GBV-C RNA was detected in four (3%), and only one of them was among the 76 patients without markers of HCV or HBV infection. Hence, the contribution of GBV-C to non-A to E hepatitis in Nepal, if any, would be small (less than 2%).

Of the 41 patients with chronic renal failure, GBV-C RNA was detected in three of the 22 (14%) on maintenance hemodialysis, but not in any of the remaining 19 without hemodialysis. Transfusions were given to the 22 patients on hemodialysis, but to none of the 19 who had not received hemodialysis. Of the 22 patients on maintenance hemodialysis, the three with GBV-C RNA had received more units of transfusion than the 19

TABLE IV. Genotypes of GBV-C in Reference to HCV Genotypes in Drug Addicts

HCV genotypes	N	GBV-C genotypes G1	G2	G3	G2/G3	GBV-C RNA (−)
I/1a	21	2 (10%)	9 (43%)	1 (5%)	1 (5%)	8 (38%)
V/3a	19	0	7 (37%)	0	0	12 (63%)
I/1a plus V/3a	2	0	2 (100%)	0	0	0
Unclassified	1	0	0	0	0	1 (100%)
HCV RNA (−)	29	0	8 (28%)	2 (7%)	0	19 (66%)
Total	72	2 (3%)	26 (36%)	3 (4%)	1 (1%)	40 (56%)

without GBV-C RNA (51 ± 21 vs. 5 ± 3 units, $P < 0.01$). Therefore, transfusion was the main source of GBV-C infection in the patients on maintenance hemodialysis in Nepal. Aside from transfusions, nosocomial transmission of GBV-C can occur through shared dialysis equipment among hemodialysis patients, which can be verified by sequence analysis of GBV-C isolates infecting them [Masuko et al., 1996; Tsuda et al., 1996; Wang et al., 1997].

The results of this study indicate that GBV-C would be prevalent even in a segregated part of the world and highlighted populations at increased risk for infection such as drug addicts and hemodialysis patients in Nepal.

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